

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims

1. (currently amended) A method of identifying a compound that is an inhibitor of mitosis comprising the steps of:

- (a) providing a kinase reaction mixture comprising a purine nucleoside triphosphate, a Nercc1 kinase protein (SEQ ID NO:2), and a kinase substrate, wherein said kinase substrate is a polypeptide that comprises a domain that is susceptible to phosphorylation by said Nercc1 kinase protein.
- (b) incubating said kinase reaction mixture in the presence and absence of a test compound for a time sufficient to permit the Nercc1 kinase protein to phosphorylate the kinase substrate, and
- (c) determining the level of phosphorylated kinase substrate in the presence and absence of said test compound,

wherein a lower level of phosphorylated kinase substrate produced in the presence of said test compound compared to the level produced in the absence of said test compound indicates that said test compound is an inhibitor of mitosis.

2. (currently amended) The method according to Claim 1, wherein the Nercc1 kinase protein is ~~initially~~ a non-activated Nercc1 kinase protein capable of auto-activation by auto-phosphorylation.

3. (currently amended) The method according to Claim 1, wherein the Nercc1 kinase protein and the kinase substrate are ~~an initially~~ a non-activated Nercc1 kinase protein or fusion protein thereof, wherein said ~~initially~~ non-activated Nercc1 kinase or fusion protein is capable of auto-activation by auto-phosphorylation.

4. (withdrawn) The method according to Claim 1, wherein the Nercc1 kinase protein of the method is an activated Nercc1 kinase.

5. (withdrawn) The method according to Claim 4, wherein the activated Nercc1 kinase is selected from the group consisting of a phosphorylated Nercc1 kinase; a recombinantly produced, activated Nercc1 kinase; a constitutively active mutant variant of Nercc1; and a fusion protein thereof.

6. (withdrawn) The method according to Claim 5, wherein said activated Ncc1 kinase is a constitutively active mutant variant of Ncc1 or a fusion protein thereof.
7. (withdrawn) The method according to Claim 6, wherein said constitutively active mutant variant of Ncc1 or fusion protein thereof is constitutively active owing to the absence of all or a portion of an RCC1 auto-inhibitory domain.
8. (currently amended) The method according to Claim 1 or Claim 2, wherein the kinase substrate is selected from the group consisting of a non-activated Ncc1 kinase protein, a non-activated Nek6 protein, a non-activated Nek7 protein (SEQ ID NO:6), a histone, a casein, a myelin basic protein (MBP), and a fusion protein thereof.
9. (withdrawn) The method according to Claim 8, wherein the kinase substrate is a histone or a fusion protein comprising a histone.
10. (withdrawn) The method according to Claim 9, wherein said histone is histone H3 or histone H4.
11. (original) The method according to Claim 1 or Claim 2, wherein said purine nucleoside triphosphate comprises an adenosine triphosphate or a guanosine triphosphate.
12. (original) The method according to Claim 1 or Claim 2, wherein said purine nucleoside triphosphate is radio-labeled with ³²P.
13. (original) The method according to Claim 1 or Claim 2, wherein step (c) of the method for determining the level of phosphorylated kinase substrate is carried out using an antibody that specifically binds the phosphorylated form of the kinase substrate.
14. (original) The method according to Claim 13, wherein said antibody specifically binds a phosphorylated kinase substrate selected from the group consisting of a phosphorylated Ncc1 kinase protein, a phosphorylated Nek6 protein, a phosphorylated Nek7 protein, a phosphorylated histone, a phosphorylated casein, a phosphorylated MBP, and a fusion protein thereof.
15. (original) The method according to Claim 1 or 2, wherein at least one step of said method is carried out in a vessel comprising a test tube, a microtiter plate, a biochip, a coverslip, and combinations thereof.

16. (original) The method according to Claim 1 or 2, wherein at least one step is carried out automatically or semi-automatically.
17. (withdrawn) The method according to Claim 1 or Claim 2, comprising the additional step:
- (d) determining whether said test compound of step (c) inhibits mitosis in dividing cells.
18. (withdrawn) The method according to Claim 17, wherein said step (d) comprises contacting dividing cells in a culture with said test compound and assaying the cells a response selected from the group consisting of cell lysis, apoptosis, disruption of mitotic spindles, misalignment of chromosomes, and combinations thereof.
19. (withdrawn) The method according to Claim 17, wherein said dividing cells are cancer cells.
20. (withdrawn) The method according to Claim 17, wherein said step (d) is carried out in a vessel selected from the group consisting of a test tube, a microtiter plate, a biochip, a coverslip, and combinations thereof.
21. (withdrawn) The method according to Claim 17, wherein said step (d) is carried out automatically or semi-automatically.
22. (withdrawn) A method of identifying an inhibitor of mitosis comprising:
- (a) providing a kinase reaction mixture comprising an activated Nek6 or Nek7 kinase protein, a kinase substrate, and a purine nucleoside triphosphate,
 - (b) incubating said reaction mixture in the presence and absence of a test compound for a time sufficient to permit the activated Nek6 or Nek7 kinase protein to phosphorylate said kinase substrate, and
 - (c) detecting the level of phosphorylated kinase substrate in the presence and absence of said test compound,
- wherein a lower level of phosphorylated kinase substrate produced in the presence of said test compound compared to the level produced in the absence of said test compound indicates that said test compound is an inhibitor of mitosis.

23. (withdrawn) The method according to Claim 22, wherein said kinase substrate is Cdc16, MBP, or a fusion protein thereof.
24. (withdrawn) The method according to Claim 22, wherein said purine nucleoside triphosphate is an adenosine triphosphate or a guanosine triphosphate.
25. (withdrawn) The method according to Claim 22, wherein said purine nucleoside triphosphate is radio-labeled with ^{32}P .
26. (withdrawn) The method according to Claim 22, wherein the step of determining the level of phosphorylated substrate kinase in step (c) of the method is carried out using an antibody that specifically binds the phosphorylated substrate kinase.
27. (withdrawn) The method according to Claim 26, wherein said antibody specifically binds a phosphorylated substrate kinase selected from the group consisting of a phosphorylated Cdc16, a phosphorylated MBP, and a fusion protein thereof.
28. (withdrawn) The method according to Claim 22, wherein at least one step is carried out in a vessel selected from the group consisting of: a test tube, a microtiter plate, a biochip, a coverslip, and combinations thereof.
29. (withdrawn) The method according to Claim 22, wherein at least one step is carried out automatically or semi-automatically.
30. (withdrawn) The method according to Claim 22, comprising the additional step:
 (d) determining whether said test compound inhibits mitosis in dividing cells.
31. (withdrawn) The method according to Claim 30, wherein step (d) comprises contacting dividing cells in a culture with said test compound and assaying the cells for a response selected from the group consisting of cell lysis, apoptosis, disruption of mitotic spindles, misalignment of chromosomes, and combinations thereof.
32. (withdrawn) The method according to Claim 31, wherein said dividing cells are cancer cells.

33. (withdrawn) The method according to Claim 30, wherein step (d) is carried out in a vessel comprising a test tube, a microtiter plate, a biochip, a coverslip, and combinations thereof.

34. (withdrawn) The method according to Claim 30, wherein step (d) is carried out automatically or semi-automatically.

35. (withdrawn) A method of diagnosing a cancerous or potentially cancerous state in an individual comprising:

- (a) assaying cells from said individual for the level of Nercc1, Nek6, or Nek7 kinase protein or corresponding kinase activity,
- (b) comparing the level of Nercc1, Nek6, or Nek7 protein or corresponding kinase activity determined in the cells from step (a) to the level determined in normal, non-cancerous cells or to the level determined in a prior sample of cells from said individual,

wherein an elevation in the level of Nercc1, Nek6, or Nek7 protein or corresponding kinase activity determined in the cells from step (a) relative to the level determined in normal, non-cancerous cells or relative to the level determined in a prior sample of cells from said individual is diagnostic for a cancerous or potentially cancerous state.

36. (withdrawn) The method according to Claim 35, wherein the level of Nercc1, Nek6, or Nek7 in step (a) is detected by a method selected from the group consisting of a kinase phosphate transfer reaction, an immunodection assay, and a transcription assay for mRNA encoding Nercc1, Nek6, or Nek7.

37. (withdrawn) The method according to Claim 35, wherein the cells of an individual in step (a) are obtained from a source selected from the group consisting of a tissue biopsy, blood, cell smear, tissue swab, bodily fluid, and feces.

38. (withdrawn) A mutant variant Nercc1 kinase protein or fusion protein thereof that is constitutively active, wherein said mutant variant Nercc1 kinase protein or fusion protein thereof is active in the absence of phosphorylation at an activation site in said mutant variant Nercc1 kinase protein or fusion protein thereof.

39. (withdrawn) The mutant variant Nercc1 kinase protein or fusion protein thereof according to Claim 38, wherein said mutant variant Nercc1 kinase protein or fusion protein thereof lacks all or a portion of an RCC1 auto-inhibitory domain.

40. (withdrawn) A mutant variant Nercc1 protein lacking all or a sufficient portion of an RCC1 auto-inhibitory domain so that said mutant variant Nercc1 protein is permanently activated to provide a functional Nercc1 kinase activity without the necessity of undergoing phosphorylation.
41. (withdrawn) The mutant variant Nercc1 protein according to Claim 41, wherein said mutant variant Nercc1 protein is Nercc1 (Δ 347-732).
42. (withdrawn) A mutant variant of Nercc1 selected from the group consisting of Nercc1 (Δ 347-732), Nercc (Δ 763-889), Nercc1 (347-732), Nercc (338-739), and a fusion protein thereof.
43. (withdrawn) A fusion protein comprising a non-Nercc polypeptide linked in frame with a mutant variant of Nercc1 selected from the group consisting of Nercc1 (Δ 347-732), Nercc (Δ 763-889), Nercc1 (347-732), and Nercc (338-739).
44. (withdrawn) The fusion protein according to any one of Claims 38, 39, 42, and 43, wherein said fusion protein comprises a non-Nercc polypeptide selected from the group consisting of glutathione S-transferase (GST), an epitope tag polypeptide, and combinations thereof.
45. (withdrawn) The fusion protein according to Claim 44, wherein said epitope tag polypeptide is selected from the group consisting of FLAG, HA, myc, and combinations thereof.